

***Pythium* and *Phytophthora* associated with
root disease of hydroponic lettuce**

Khalaf Alhussaen

Doctor of Philosophy

University of Technology Sydney

2006

DECLARATION

The work presented in this thesis is original and contains no material formerly published or written by another person, except where due acknowledgement has been specified. I hereby declare that I have not submitted this material to any institution for a degree or diploma.

Khalaf Alhussaen



Sydney

June 2006

ACKNOWLEDGEMENTS

The achievement of this study would not have been possible without the kind support that I have received from many people. I owe an enormous debt of gratitude to all of them.

First, I would like to deeply thank my principal supervisor, Dr Jane Tarran, for her support in all aspects of this project during the last three years; words will not be enough to thank her. I would also like to thank my co-supervisors Dr Brett Summerell (Botanic Gardens Trust), Dr Fraser Torpy (University of Technology Sydney) and Mr Len Tesoriero (NSW Department of Primary Industries), for suggestions and support during my study.

I would like to thank the Botanic Gardens Trust Sydney for their support, providing a friendly working environment, including people in the Plant Pathology Laboratory. In particular, I would like to thank Suzanne Bullock, Linda Gunn, Julie Bates, Chris Howard, Sophie Peterson and Ratiya Pongpisutta. They have encouraged me, advised me on techniques, and offered many suggestions and comments on my work. Also, I would like to thank Dr Chris Allen for his help with the preparation of the maps and people in the nursery, especially Stephen Bartlett.

I would also like to thank the University of Technology Sydney, Faculty of Science, Department of Environmental Sciences, for their support during my project. At the University of Technology Sydney, I would like to thank specific people who supported me and my research during the last three years - Professor Derek Eamus, Rochelle Seneviratne, Narelle Richardson, Christine Wojak, Gemma Armstrong, Sue Fenech, Peter Jones, Marnie Robinson, Ed Soliman and Tony Ye. I would also like to thank the staff of the Gore Hill library and Jamie Gabriel from the fees office.

I would like to thank many individuals and institutions who helped me with my project, particularly Manicaro's Hydroponic Lettuce Farm, Camilleri's Farm, Borg Hydroponics, Pacific Hydroponics, Speedy Seedlings® and Supplies Pty Ltd, the Bureau of Meteorology NSW and Terranova Seeds Pty Ltd. Also, many thanks to Professor Lester

Burgess at the University of Sydney and his group (Fusarium Research Lab) for allowing me to participate their weekly research meetings.

I would like to thank many friends, particularly Dr Hyder Aldomi, Dr Hosam Malkawi, Dr Mohammad Shatnawi, Mahmud Malkawi and others.

Finally, I would like to thank my family, especially my parents and my older brother for continued support, both emotionally and financially by giving me a scholarship from their personal finances.

SUMMARY

Root rot disease of lettuce grown hydroponically has become a serious problem in Australia and worldwide. Farmers in Australia claim that they have suffered heavy yield losses of hydroponic lettuce in summer in recent years. The research reported in this thesis focused on root disease of lettuce grown in hydroponic systems in the Sydney area and included determination of the causes of this disease, isolation of pathogenic organisms and identification of pathogens by using morphological and physiological studies, as well as molecular techniques. The technique involving Inter Simple Sequence Repeats (ISSR) was also used to study the relationships among the populations of these pathogens. Moreover, the effect of the temperature of the nutrient solution on disease development was also investigated. The research reported here represents the first comprehensive survey of hydroponic lettuce farms in and near Sydney, New South Wales (NSW) in relation to root disease.

Two surveys investigated root disease severity on lettuce grown in hydroponic systems in the Sydney and Central Coast areas of NSW. Three different lettuce cultivars (Baby Cos, Red Oak and Brown Mignonette) were surveyed five times over an 11 month period (May 2003 to March 2004) in one farm (Leppington 1) in the first survey. In the second survey, four different lettuce cultivars (Baby Cos, Red Oak, Green Oak and Brown Mignonette) were surveyed five times over an 11 month period (May 2004 to March 2005) in four different farms (Leppington 1 and 2 and Central Coast 1 and 2). From these two surveys, it appears that root disease of hydroponic lettuce occurred at farms only in the warmer times of the year, when the nutrient solution temperature was 20-30.5°C, and not in the cooler times of the year, when the nutrient solution temperature was 13.5-18°C.

In order to isolate pathogenic organisms, lettuce plants were sampled during the two farm-based surveys. Isolations were carried out from the roots of the cultivars surveyed from the same farms and at the same times as the surveys. Two genera of oomycetes, *Pythium* (81 isolates) and *Phytophthora* (68 isolates), were the main microorganisms isolated from lettuce roots grown in hydroponic systems. *Pythium* was isolated all year round (from 60-100% of samples), but a disease problem at the farms only occurred in

the months with higher temperatures (November, January and March). *Phytophthora* was isolated nearly all year around (from 19-80% of samples).

Isolates of *Pythium* and *Phytophthora* were generally found to be pathogenic to lettuce plants at 25°C and 35°C, but not at 15°C, when lettuce were grown in potting mix. *Pythium coloratum* was found to be pathogenic to lettuce plants grown in an experimental hydroponic system when the nutrient solution temperature was between 22°C and 26°C. Other fungi, such as *Fusarium* spp. and *Rhizoctonia* spp., were also isolated but only infrequently and they were not associated with root disease in the farm at the time of isolation. Furthermore, they were not pathogenic to lettuce grown in potting mix at 15, 25 or 35°C.

The effects of the temperature of the nutrient solution on root disease of lettuce caused by *Pythium* and *Phytophthora* were examined in an experimental hydroponic system. Root rot disease occurred following inoculation with an isolate belonging to *Pythium* group F, or a combination of this isolate and *Phytophthora drechsleri*, under a temperature regime of 24-27°C but not at 16-17°C. Yield reduction was found in plants inoculated with an isolate belonging to *Pythium* group F, *Phytophthora drechsleri* and a combination of the two, at a nutrient solution temperature regime which involved exposure to 34°C for 10 hours, followed by 18-20°C for the remainder of the experiment.

Morphological features and physiological characteristics were used to identify 81 isolates of *Pythium* and 68 isolates of *Phytophthora* obtained from roots of hydroponic lettuce. Molecular techniques were also used for identification including polymerase chain reaction-random fragment length polymorphisms (PCR-RFLP) and sequencing of the internal transcribed spacer (ITS) region of rDNA. For population studies, the ISSR technique was used. The 81 isolates of *Pythium* could be divided into three groups on the basis of colony characteristics. Eighty *Pythium* isolates were identified as belonging to *Pythium* group F and one isolate as *Pythium coloratum*. All 68 *Phytophthora* isolates were identified as *Phytophthora drechsleri*. The optimum growth temperature of the isolates belonging to *Pythium* group F and the isolate of *Pythium coloratum* was 30°C. They also grew well at temperatures of 25°C and 35°C and could still grow at 40°C and 5°C. The optimum growth temperature for the isolates of *Phytophthora drechsleri* was

25°C but they were still able to grow at temperatures of 10°C and 35°C. An assessment of mating type was used as a biological marker for all *Phytophthora drechsleri* isolates. All isolates were found to be heterothallic and of the A₁ mating type. They produced oogonia with amphigynous antheridia when paired with the A₂ mating type of *Phytophthora cryptogea*.

When 81 *Pythium* isolates were examined using four primers with the ISSR technique, 11 groups were established. A slight correlation was found between the groups and the sampling times at which isolates in the groups were obtained. However, no correlations were found between the groups and either the farm or the geographic area from which isolates were obtained. Furthermore, there was no correlation between these groups and the lettuce cultivars yielding the isolates. Moreover, no correlations were found between the groups established by the ISSR technique and the three groups identified on the basis of colony characteristics.

The ISSR technique applied to *Phytophthora* isolates yielded six groups. A correlation was found between these groups and the sample times at which isolates were obtained, on the basis of cooler season samples (May and August together) compared with warmer season samples (November, January and March together). No correlation was found between the groups and either the farms or the geographic areas from which the isolates were obtained. Furthermore, there was no correlation between the groups and the lettuce cultivars yielding the isolates.

Based on the findings of this research, root rot disease management in hydroponic lettuce could be achieved by reducing the temperature of the nutrient solution in summer to 20°C or less, whilst maintaining it within a range favourable to lettuce growth. Moreover, methods to reduce the inoculum level of *Pythium* (and possibly *Phytophthora* as well) are worth investigating, as are methods of disease management based on biological control.

TABLE OF CONTENTS

Declaration.....	i
Acknowledgements.....	ii
Summary.....	iv
list of Figures	xii
List of Tables	xv
List of abbreviations	xviii

1 General introduction and literature review	1
1.1 Introduction	1
1.2 Hydroponics as a plant production system	2
1.2.1 Overview of hydroponics.....	2
1.2.2 Advantages and disadvantages of hydroponic systems.....	3
1.2.3 Types of hydroponic systems.....	4
1.2.4 Nutrient Film Technique.....	5
1.3 Lettuce	6
1.3.1 General aspects	6
1.3.2 Types of lettuce.....	6
1.3.2.1 Crisp Head	6
1.3.2.2 Butter Head	7
1.3.2.3 Leaf Lettuce or Loose Leaf.....	7
1.3.2.4 Oak Leaf	7
1.3.2.5 Cos or Romaine	7
1.4 Lettuce diseases	8
1.4.1 Bacteria.....	8
1.4.2 Nematodes	9
1.4.3 Viruses	9
1.4.4 Fungi.....	9
1.4.5 Oomycetes	10
1.4.6 Abiotic or non-infectious diseases	12
1.5 Environmental conditions and disease development.....	12
1.6 The phylum Oomycota (<i>Pythium</i> spp. and <i>Phytophthora</i> spp.)	15
1.6.1 <i>Pythium</i> species	16
1.6.2 <i>Phytophthora</i> species.....	17
1.7 Identification of <i>Pythium</i> spp. and <i>Phytophthora</i> spp.	18
1.7.1 <i>Pythium</i> species	18
1.7.2 <i>Phytophthora</i> species.....	21
1.8 Aims of the research	23
2 Survey of root rot disease in hydroponic lettuce in the Sydney area, isolation of fungi and oomycetes, and their temperature responses in culture	25
2.1 Introduction	25
2.2 Materials and methods.....	29
2.2.1 Root rot disease survey.....	29
2.2.2 Lettuce sampling, isolation of fungi and oomycetes from lettuce roots and identification of these microorganisms	32
2.2.3 Pathogenicity tests	34
2.2.4 Temperature responses in culture of fungi and oomycetes	37
2.2.5 Data analysis	38
2.3 Results	39
2.3.1 Root rot disease survey.....	39
2.3.2 Isolation of fungi and oomycetes from lettuce roots and identification of these microorganisms.....	43
2.3.3 Pathogenicity tests	46

2.3.4	Temperature responses in culture of fungi and oomycetes	52
2.4	Discussion.....	55
3	Survey of root rot disease of lettuce grown in hydroponic systems in Sydney and the central coast.....	62
3.1	Introduction	62
3.2	Materials and methods.....	64
3.2.1	Root rot disease survey.....	64
3.2.2	Isolation and identification of oomycetes from lettuce roots.....	68
3.2.3	Pathogenicity tests	69
3.2.3.1	Hydroponic units.....	69
3.2.3.2	Plant materials.....	70
3.2.3.3	Inoculation	70
3.2.3.4	Data collection	71
3.2.4	Data analysis	71
3.3	Results	73
3.3.1	Root rot disease survey.....	73
3.3.1.1	Baby Cos.....	74
3.3.1.2	Red Oak	77
3.3.1.3	Green Oak	80
3.3.1.4	Brown Mignonette	83
3.3.2	Isolation and identification of oomycetes from lettuce roots.....	86
3.3.3	Pathogenicity tests	89
3.4	Discussion.....	92
4	Effects of Temperature on root rot disease of hydroponic lettuce	96
4.1	Introduction	96
4.2	Materials and methods.....	99
4.2.1	Hydroponic system	99
4.2.2	Experimental design, glasshouse conditions and nutrient solution temperatures	99
4.2.3	Plant materials	100
4.2.4	Inoculum preparation and inoculation	100
4.2.5	Data collection	101
4.2.6	Data analysis.....	101
4.3	Results	103
4.3.1	Nutrient solution temperature 24-27°C.....	103
4.3.2	Nutrient solution temperature 34°C.....	106
4.3.3	Nutrient solution temperature 16-17°C.....	109
4.3.4	Nutrient solution temperature 34°C for 10 h followed by 18-20°C.....	113
4.4	Discussion.....	116
5	Characterisation and Identification of <i>Pythium</i> spp. Isolated from lettuce roots by morphological and molecular techniques	121
5.1	Introduction	121
5.2	Materials and methods.....	126
5.2.1	Isolation of <i>Pythium</i> spp. from lettuce roots	126
5.2.1.1	Recovery of <i>Pythium</i> cultures	126
5.2.1.2	Hyphal tip isolation.....	126
5.2.2	Morphological studies.....	126
5.2.2.1	Colony characteristics on different agar media.....	126
5.2.2.2	Morphological characteristics using light microscopy.....	127
5.2.3	Physiological studies.....	128
5.2.3.1	Colony growth rates at different temperatures and optimum, minimum and maximum temperatures.....	128
5.2.4	Molecular studies.....	128
5.2.4.1	DNA extraction	128
5.2.4.2	Gel electrophoresis of genomic DNA	129

5.2.4.3	PCR amplification of ITS region of ribosomal DNA.....	129
5.2.4.4	Restriction enzyme digestion for restriction fragment length polymorphism (RFLP) analysis	130
5.2.4.5	Sequencing the ITS region of ribosomal DNA	131
5.2.4.6	Inter Simple Sequence Repeat (ISSR) analysis for population studies	132
5.3	Results	134
5.3.1	Recovery of <i>Pythium</i> spp. cultures	134
5.3.2	Morphological studies.....	137
5.3.2.1	Colony characteristics on different agar media.....	137
5.3.3	Morphological characteristics using light microscopy	139
5.3.3.1	Group (i)	139
5.3.3.2	Group (ii)	141
5.3.3.3	Group (iii)	142
5.3.4	Physiological studies.....	143
5.3.4.1	Colony growth rates at different temperatures and optimum, minimum and maximum temperatures.....	143
5.3.5	Molecular studies.....	145
5.3.5.1	Comparison of isolates by PCR-RFLP of ITS region of rDNA.....	145
5.3.5.2	Sequence of ITS region of rDNA.....	147
5.3.5.3	Variation among populations of <i>Pythium</i> isolates by using Inter Simple Sequence Repeat (ISSR) analysis	148
5.3.5.3.1	Variation observed using the AC-primer.....	148
5.3.5.3.2	Variation observed using the AG-primer.....	150
5.3.5.3.3	Variation observed using the CGA-primer.....	152
5.3.5.3.4	Variation observed using the GT-primer.....	154
5.3.5.3.5	Variation observed using the four ISSR primers	156
5.3.6	Storage of representative isolates.....	156
5.4	Discussion.....	158

6 Characterisation and Identification of *Phytophthora* spp. Isolated from lettuce roots by morphological and molecular techniques..... 164

6.1	Introduction	164
6.2	Materials and methods.....	168
6.2.1	Isolation of <i>Phytophthora</i> spp. from lettuce roots	168
6.2.1.1	Recovery of <i>Phytophthora</i> cultures	168
6.2.1.2	Hyphal tip isolation.....	168
6.2.2	Molecular studies.....	169
6.2.2.1	DNA extraction.....	169
6.2.2.2	Gel electrophoresis of genomic DNA	169
6.2.2.3	PCR amplification of ITS region of ribosomal DNA.....	169
6.2.2.4	Restriction enzyme digestion for restriction fragment length polymorphism (RFLP) analysis	169
6.2.2.5	Sequencing the ITS region of ribosomal DNA	170
6.2.2.6	Inter Simple Sequence Repeat (ISSR) analysis for population studies.....	170
6.2.3	Morphological studies.....	171
6.2.3.1	Colony characteristics on different agar media.....	171
6.2.3.2	Morphological characteristics using light microscopy.....	171
6.2.4	Physiological studies.....	171
6.2.4.1	Colony growth rates at different temperatures and optimum, minimum and maximum temperatures.....	171
6.2.5	Determination of mating type	172
6.3	Results	173
6.3.1	Recovery of <i>Phytophthora</i> cultures	173
6.3.2	Molecular studies.....	176
6.3.2.1	Comparison of isolates by PCR-RFLP of ITS region of rDNA.....	176
6.3.2.2	Sequence of ITS region of rDNA.....	178
6.3.2.3	Variation among populations of <i>Phytophthora drechsleri</i> by using Inter Simple Sequence Repeat (ISSR) analysis	179
6.3.2.3.1	Variation observed using the AC-primer.....	179

6.3.2.3.2	Variation observed using the AG-primer.....	181
6.3.2.3.3	Variation observed using the CGA-primer.....	183
6.3.2.3.4	Variation observed using the GT-primer.....	185
6.3.2.3.5	Variation observed using the four ISSR primers.....	187
6.3.3	Morphology studies.....	189
6.3.3.1	Colony characteristics on different agar media.....	189
6.3.3.2	Morphological characteristics.....	190
6.3.4	Physiological studies.....	192
6.3.4.1	Colony growth rates at different temperatures and optimum, minimum and maximum temperatures.....	192
6.3.5	Mating type.....	193
6.3.6	Storage of representative isolates.....	194
6.4	Discussion.....	195
7	General discussion	200
8	References.....	208
9	Appendix.....	225
1.	Growth media	226
2.	Nutrient solution for growth of lettuce plants	228
3.	Data analysis for Chapter 2: Survey of root rot disease in hydroponic lettuce in the Sydney area, isolation of fungi and oomycetes, and their temperature responses in culture.	229
a.	Root assessment (section 2.3.1).....	229
b.	Isolation (section 2.3.2)	235
c.	Pathogenicity tests (section 2.3.3)	237
1.	<i>Pythium</i> Pathogenicity tests	237
2.	<i>Phytophthora</i> sp. pathogenicity tests (section 2.3.3) (analysis for Table 2-8).....	255
3.	<i>Fusarium avenaceum</i> pathogenicity tests (section 2.3.3) (analysis for Table 2-9).....	261
4.	<i>Fusarium oxysporum</i> pathogenicity tests (section 2.3.3) (analysis for Table 2-9).....	262
5.	<i>Rhizoctonia</i> sp. pathogenicity tests (section 2.3.3) (analysis for Table 2-10).....	266
6.	The combination of <i>Pythium</i> sp. and <i>Phytophthora</i> sp. (section 2.3.3) (analysis for Table 2-11).....	267
d.	Temperature responses in culture of fungi and oomycetes (section 2.3.4).....	272
4.	Data analysis for Chapter 3: Survey of root rot disease of lettuce grown in hydroponic systems in Sydney and the Central Coast.....	273
a.	Comparisons between times, farms and cultivars (section 3.3.1)	273
b.	Baby Cos (section 3.3.1.1) (analyses for Tables 3-2 and 3-3)	273
c.	Red Oak (section 3.3.1.2 and Tables 3-4 and 3-5).....	279
d.	Green Oak (section 3.3.1.3) (analysis for Table 3-6 and 3-7).....	285
e.	Brown Mignonette (section 3.3.1.4) (analysis for Table 3-8 and 3-9).....	291
f.	Isolation (section 3.3.2)	296
g.	Pathogenicity tests (section 3.3.3)	298
5.	Data analysis for Chapter 4: Effects of temperature on root rot disease of hydroponic lettuce	300
a.	Nutrient solution temperature 24-27°C (section 4.3.1).....	300
b.	Nutrient solution temperature 34°C continuously heated (section 4.3.2)	302
c.	Nutrient solution temperature 16-17°C (section 4.3.3).....	305
d.	Nutrient solution temperature 34°C for 10 h followed by 18-20°C.....	308
e.	Typical air temperatures in the glasshouse (measured using Tiny Tags) during experiments at each of four temperature regimes.	311
6.	Solution for DNA extraction and molecular biology reagents	313
7.	Data analysis for Chapter 5: Characterisation and identification of <i>Pythium</i> spp. isolated from lettuce roots by morphological and molecular techniques.	315

a.	<i>Pythium</i> growth at different temperatures (section 5.3.4.1 and Table 5-5).....	315
b.	<i>Pythium</i> sequences (section 5.3.5.2 and Table 5-6)	317
c.	Representative isolates of the 81 isolates of <i>Pythium</i> obtained in the present study stored in the collection of the Agricultural Institute at NSW Agriculture Department (Orange, NSW, Australia) (DAR number).....	320
8.	Data analysis for Chapter 6: Characterisation and identification of <i>Phytophthora</i> spp. isolated from lettuce roots by morphological and molecular techniques.	321
a.	<i>Phytophthora</i> sequences (section 6.3.2.2 and Table 6-4)	321
b.	Morphological characteristics (section 6.3.3.2)	322
c.	<i>Phytophthora</i> growth at different temperatures (section 6.3.4.1)	327
d.	Representative isolates of 68 isolates of <i>Phytophthora drechsleri</i> obtained in the present study in the collection of the Agricultural Institute at NSW Agriculture Department (Orange, NSW, Australia) (DAR number).....	328

LIST OF FIGURES

Figure 1-1 Nutrient Film Technique (NFT) for lettuce production. (Manicaró's Lettuce Farm, Leppington, NSW.)	5
Figure 1-2 Different lettuce cultivars grown in the NFT system in the Sydney area.	8
Figure 1-3 <i>Pythium</i> sp. a: hyphae and b: diclinous antheridia.....	16
Figure 1-4 <i>Phytophthora</i> sp. a: hyphae; b: sporangium; c: globose oogonium with amphigynous antheridium.....	17
Figure 1-5 The Internal Transcribed Spacer (ITS) region of ribosomal DNA (rDNA)	19
Figure 1-6 Phylogenetic tree of <i>Pythium</i> isolates from this study [Scott <i>et al.</i> 2005] and previously characterised <i>Pythium</i> spp. based on DNA sequence analysis of the region encompassing ITS I, 5.8S rDNA and ITS II. The numbers at each branch point are bootstrap values following resampling of the data 1000 times. [This study was based on 130 isolates of <i>Pythium</i> from the Lockyer Valley, Queensland (LVP), which were grouped into three main groups (A, B and C) and three minor groups (D, E and F).].....	20
Figure 2-1 Manicaró's Hydroponic Lettuce Farm (216 Rickard Road, Leppington, NSW), surveyed from May 2003 to March 2004.	30
Figure 2-2 Map of the Sydney area showing Leppington (location of Manicaró's Lettuce Farm), Flemington (location of main Sydney fruit and vegetable market) and the Sydney Central Business Direct (CBD).	31
Figure 2-3 Lettuce cultivars examined for root rot diseases.	31
Figure 2-4 Scale used to assess root rot disease severity on hydroponic lettuce roots:.....	32
Figure 2-5 Mean monthly maximum air temperature at Leppington, NSW (Bureau of Meteorology, NSW, station 67020, mean of daily maxima) from April 2003 to March 2004, hydroponic nutrient solution temperature on five survey days from May 2003 to March 2004 and maximum air temperature on the survey days.	39
Figure 2-6 Percentage of diseased Brown Mignonette lettuce (both mature and young) from the five time periods (May-03, Aug-03, Nov-03, Jan-04 and Mar-04) with root rot ratings of 3 and 4. Temperature of the nutrient solution was measured on the survey day.	40
Figure 2-7 Percentage of diseased Baby Cos lettuce (both mature and young) from the five time periods (May-03, Aug-03, Nov-03, Jan-04 and Mar-04) with root rot ratings of 3 and 4. Temperature of the nutrient solution was measured on the survey day. Error bars are standard error of the mean.	41
Figure 2-8 Percentage of diseased Red Oak lettuce (both mature and young) from the five time periods (May-03, Aug-03, Nov-03, Jan-04 and Mar-04) with root rot ratings of 3 and 4. Temperature of the nutrient solution was measured on the survey day.	42
Figure 2-9 Lettuce plants from November 2003 pathogenicity test involving an isolate of <i>Pythium</i> sp. tested at 25°C. Inoculated plants ((a) and (c)) show wilting of leaves (in (a)) and a small root system/brown roots (in (c)). Control plants ((b) and (d)) show no disease and a larger root system with healthy white roots.	47
Figure 2-10 Lettuce plants from November 2003 pathogenicity test involving an isolate of <i>Phytophthora</i> sp. tested at 25°C. Inoculated plants ((a) and (c)) show wilting of leaves (in (a)) and a small root system/brown roots (in (c)). Control plants ((b) and (d)) show no disease and a larger root system with healthy white roots.....	49
Figure 2-11 Mean colony radius (mm) of the main <i>Pythium</i> isolates from lettuce roots grown on PCA media and measured at 24h and 48h after inoculation and incubation in the dark at nine temperatures from 5°C to 45°C. Bars are standard error of the mean.	52
Figure 2-12 Mean colony radius (mm) of the main <i>Phytophthora</i> isolates from lettuce roots grown on PCA media and measured at 24h, 48h and 120h after inoculation and incubation in the dark at nine temperatures from 5°C to 45°C.	53
Figure 2-13 Mean colony radius (mm) of the main <i>Fusarium oxysporum</i> isolates from lettuce roots grown on PCA media and measured at 24h, 48h and 120h after inoculation and incubation in the dark at nine temperatures from 5°C to 45°C.	53
Figure 2-14 Mean colony radius (mm) of the main <i>Fusarium avenaceum</i> isolates from lettuce roots grown on PCA media and measured at 24h, 48h and 120h after inoculation and incubation in the dark at nine temperatures from 5°C to 45°C.	54
Figure 3-1 Map of Sydney area and Central Coast of NSW. Two farms were surveyed in the Leppington area (South-west Sydney) and two at Warnervale/ Lake Munmorah area (Central Coast). The main fruit and vegetable market is located at Flemington not far from the Sydney CBD.....	65

Figure 3-2 The four hydroponic lettuce farms surveyed in this study.....	65
Figure 3-3 The four lettuce cultivars surveyed and sampled.	67
Figure 3-4 Scale used to assess root rot disease severity on hydroponic lettuce roots:.....	67
Figure 3-5 Scale of one to four used to assess disease symptoms on lettuce leaves affected by root rot pathogens:	68
Figure 3-6 Mean monthly maximum air temperature in the Leppington area (station 67020) and Central Coast area (station 61351) of NSW from April 2004 to March 2005 (Bureau of Meteorology) and mean hydroponic nutrient solution temperature on five survey days from the both farms in the two areas.....	73
Figure 3-7 Disease severity on roots of Baby Cos lettuce cultivar from the four farms surveyed five times during the 11 month period as indicated by disease index (DI) based on a scale of 1 to 4.....	75
Figure 3-8 Leaf symptom assessment for Baby Cos lettuce cultivar from the four farms surveyed five times during the 11 month period as indicated by disease index (DI) based on a scale of 1 to 4.....	76
Figure 3-9 Disease severity on roots of Red Oak lettuce cultivar from the four farms surveyed five times during the 11 month period as indicated by disease index (DI) based on a scale of 1 to 4.....	78
Figure 3-10 Leaf symptom assessment for Red Oak lettuce cultivar from the four farms surveyed five times during the 11 month period as indicated by disease index (DI) based on a scale of 1 to 4.....	79
Figure 3-11 Disease severity on roots of Green Oak lettuce cultivar from the four farms surveyed five times during the 11 month period as indicated by disease index (DI) based on a scale of 1 to 4.....	81
Figure 3-12 Leaf symptom assessment for Green Oak lettuce cultivar from the four farms surveyed five times during the 11 month period as indicated by disease index (DI) based on a scale of 1 to 4.....	82
Figure 3-13 Disease severity on roots of Brown Mignonette lettuce cultivar from the four farms surveyed five times during the 11 month period as indicated by disease index (DI) based on a scale of 1 to 4.	84
Figure 3-14 Leaf symptom assessment for Brown Mignonette lettuce cultivar from the four farms surveyed five times during the 11 month period as indicated by disease index (DI) based on a scale of 1 to 4.	85
Figure 3-15 Pathogenicity test involving an isolate of <i>Pythium</i> obtained from farm CC2 in March 2005 that appeared different from all previous isolates of <i>Pythium</i>	90
Figure 3-16 Disease severity on roots of Brown Mignonette cultivar inoculated with <i>Pythium</i> sp. and non-inoculated as control using a scale of 1 to 4: (1) healthy white roots; (2) generally healthy white roots, but with some brown colouration; (3) unhealthy roots, most roots brown in colour and (4) dead roots and/or black roots (Figure 3-4). Error bars are the standard error of the mean....	90
Figure 4-1 Root rot disease on Brown Mignonette lettuce cultivar grown in an experimental hydroponic system under a temperature regime of 24-27°C for 21 days. Plants were inoculated with one of four treatments.....	104
Figure 4-2 Disease severity on roots of Brown Mignonette lettuce cultivar inoculated with four treatments and grown at 24-27°C temperature regime for 21 days indicated by disease index (DI) based on a scale of 1 to 4 (Figure 2-4 in Chapter 2). Error bars are standard error of the mean. For each value, n = 30.....	105
Figure 4-3 Root rot disease on Brown Mignonette lettuce cultivar grown in an experimental hydroponic system under a temperature regime of 34°C (continuously heated) for 21 days. Plants were inoculated with one of four treatments.....	107
Figure 4-4 Disease severity on roots of Brown Mignonette lettuce cultivar inoculated with four treatments and grown at 34°C (continuously heated) temperature regime for 21 days indicated by disease index (DI) based on a scale of 1 to 4 (Figure 2-4 in Chapter 2). Error bars are standard error of the mean. For each value, n = 30.	108
Figure 4-5 Root rot disease on Brown Mignonette lettuce cultivar grown in an experimental hydroponic system under a temperature regime of 16-17°C for 21 days. Plants were inoculated with one of four treatments.....	110
Figure 4-6 Root cells of Brown Mignonette lettuce cultivar grown at a nutrient solution temperature of 16-17°C. Plants were inoculated with:	111
Figure 4-7 Disease severity on roots of Brown Mignonette lettuce cultivar inoculated with four treatments and grown at 16-17°C temperature regime for 21 days indicated by disease index (DI) based on a scale of 1 to 4 (Figure 2-4 in Chapter 2). Error bars are standard error of the mean. For each value, n = 30.....	112

Figure 4-8 Root rot disease on Brown Mignonette lettuce cultivar grown in an experimental hydroponic system under a temperature regime of 34°C for 10 h followed by 18-20°C for the remainder of the 21 days. Plants were inoculated with one of four treatments.	114
Figure 4-9 Disease severity on roots of Brown Mignonette lettuce cultivar inoculated with four treatments and grown at 34°C for 10 h followed by 18-20°C temperature regime for 21 days indicated by disease index (DI) based on a scale of 1 to 4 (Figure 2-4 in Chapter 2). Error bars are standard error of the mean. For each mean value, n = 30.	115
Figure 5-1 Representative cultures of each of the three major groups of isolates of <i>Pythium</i> spp. (group (i) top row; (ii), middle row; and (iii), bottom row) grown on three different media (column 1, PCA; column 2, PDA; and column 3, CMA). Cultures were grown in the dark at 25°C ± 1°C for 5 days.	138
Figure 5-2 Structures of <i>Pythium</i> from group (i) isolates.	140
Figure 5-3 Structures of <i>Pythium</i> from group (ii) isolates.	141
Figure 5-4 Structures of <i>Pythium</i> from group (iii) isolates.	142
Figure 5-5 Mean colony growth (mm) in 24 h of 15 isolates of <i>Pythium</i> grown on PCA media at a range of temperatures from 5°C to 45°C in the dark. Colony growth was measured between 24h and 48h. Bars are standard error of the mean.	144
Figure 5-6 Representative gel of PCR amplification products of ITS region of rDNA for ten <i>Pythium</i> isolates.	145
Figure 5-7 Gel photo of digested products of ITS amplification with restriction enzymes <i>EcoRI</i> (a) and <i>XmnI</i> (b) for seven <i>Pythium</i> isolates.	146
Figure 5-8 Gel photo of digested products of ITS amplification with restriction enzymes <i>MseI</i> (c) and <i>MspI</i> (d) for seven <i>Pythium</i> isolates.	146
Figure 5-9 ISSR Profiles observed for isolates of <i>Pythium</i> using AC-primer.	149
Figure 5-10 ISSR profiles observed for isolates of <i>Pythium</i> using AG-primer.	151
Figure 5-11 ISSR profiles observed for isolates of <i>Pythium</i> using CGA-primer.	153
Figure 5-12 ISSR profiles observed for isolates of <i>Pythium</i> using GT-primer.	155
Figure 5-13 Dendrogram of ISSR similarity using Dice Coefficient based on UPGMA clustering of <i>Pythium</i> isolates from different cultivars, different sample times or different farms. Groups 1 to 11 are shown for Coefficient 0.50. See Table 5-4 for isolate codes corresponding to isolate numbers shown at right. Colours indicate sample times as follows: green=May; blue=August, yellow=November; red=January; and black=March.	157
Figure 6-1 Representative gel of PCR amplification products of ITS region of rDNA for six <i>Phytophthora</i> isolates.	176
Figure 6-2 Gel photo of digested products of ITS amplification followed by <i>MspI</i> restriction enzyme for 15 different <i>Phytophthora</i> isolates.	177
Figure 6-3 Gel photo of digested products of ITS amplification followed by <i>RsaI</i> restriction enzyme for 15 different <i>Phytophthora</i> isolates.	177
Figure 6-4 Gel photo of digested products of ITS amplification followed by <i>TaqI</i> restriction enzyme for 15 different <i>Phytophthora</i> isolates.	178
Figure 6-5 ISSR profiles observed for isolates of <i>Phytophthora drechsleri</i> using AC-primer.	180
Figure 6-6 ISSR profiles observed for isolates of <i>Phytophthora drechsleri</i> using AG-primer.	182
Figure 6-7 ISSR profiles observed for isolates of <i>Phytophthora drechsleri</i> using CGA-primer.	184
Figure 6-8 ISSR profiles observed for isolates of <i>Phytophthora drechsleri</i> using GT-primer.	186
Figure 6-9 Dendrogram of ISSR similarity using Dice Coefficient based on UPGMA clustering of <i>Phytophthora drechsleri</i> from different times, farms and lettuce cultivars. Groups 1 to 6 are shown for Coefficient 0.78. See Table 6-3 for isolate codes corresponding to isolate numbers shown at right. Colours indicate sample times as follows: green=May; blue=August, yellow=November; red=January; and black=March.	188
Figure 6-10 Colonies of the 15 <i>P. drechsleri</i> isolates tested formed a stellate to rosaceous pattern on PDA (a and b). On PCA, colonies were stellate to petallate in pattern (Figure 6-10 c), while on CMA, were colonies without any special pattern (Figure 6-10 d).	189
Figure 6-11 <i>Phytophthora drechsleri</i> hyphae (a and b) and hyphal swellings (c) on PCA.	190
Figure 6-12 A nonpapillate and noncaducous sporangium of <i>Phytophthora drechsleri</i> with different shapes.	191
Figure 6-13 Mean colony growth (mm) in 24 h for 15 isolates of <i>Phytophthora drechsleri</i> grown on PCA media at range of temperatures from 5°C to 45°C in the dark. Colony growth was measured between 24h and 48h. Bars are standard error of the mean.	193
Figure 6-14 Globose oogonia with amphigynous antheridia in <i>P. drechsleri</i>	193

LIST OF TABLES

Table 1-1 The top ten hydroponic producing countries in the world.	3
Table 1-2 Fungal diseases on lettuce plants and their symptoms	10
Table 1-3 Oomycete diseases on lettuce plants and their symptoms.....	11
Table 2-1 Fungi and oomycetes isolated from hydroponic lettuce roots obtained at five sample times and tested in pathogenicity tests.	36
Table 2-2 Fungi and oomycetes isolated from hydroponic lettuce roots obtained at five sample times and tested for temperature responses in culture.....	38
Table 2-3 Numbers of young and mature lettuce plants yielding <i>Pythium</i> spp. from their roots compared with total number of plants sampled at five times from May 2003 to March 2004. Subsequent data from May 2004 and August 2004 are also given (in italics). Nutrient solution temperature was measured on the sample day.....	44
Table 2-4 Numbers of young and mature lettuce plants yielding <i>Phytophthora</i> spp. from their roots compared with total number of plants sampled at five times from May 2003 to March 2004. Nutrient solution temperature was measured on the sample day.....	45
Table 2-5 Numbers of young and mature lettuce plants yielding <i>Fusarium</i> spp. from their roots compared with total number of plants sampled at five times from May 2003 to March 2004. Nutrient solution temperature was measured on the sample day.....	45
Table 2-6 Numbers of young and mature lettuce plants yielding <i>Rhizoctonia</i> spp. from their roots compared with total number of plants sampled at five times from May 2003 to March 2004. Nutrient solution temperature was measured on the sample day.....	45
Table 2-7 <i>P</i> value of the mean difference between control plants and plants inoculated with <i>Pythium</i> isolates for wet shoot weight, dry shoot weight, wet root weight and dry root weight from different pathogenicity test experiments (August 2003, November 2003, January 2004 and March 2004) under different temperatures (15°C, 25°C and 35°C). Means for the control plants were greater than the means for the inoculated plants. <i>P</i> values indicating significant differences ($P \leq 0.05$) are shown in bold. (Appendix 3 c 1.).....	48
Table 2-8 <i>P</i> value of the mean difference between control plants and plants inoculated with <i>Phytophthora</i> isolates for wet shoot weight, dry shoot weight, wet root weight and dry root weight from different pathogenicity test experiments (May 2003, November 2003, January 2004 and March 2004) under different temperatures (15°C, 25°C and 35°C). Means for the control plants were greater than the means for the inoculated plants. <i>P</i> values indicating significant differences ($P \leq 0.05$) are shown in bold. (Appendix 3 c 2.).....	50
Table 2-9 <i>P</i> value of the mean difference between control plants and inoculated plants for wet shoot weight, dry shoot weight, wet root weight and dry root weight from different pathogenicity test experiments (May 2003 and August 2003) under different temperatures (15°C, 25°C and 35°C) for <i>Fusarium avenaceum</i> and <i>Fusarium oxysporum</i> isolates. Means for the control plants were greater than the means for the inoculated plants. (Appendix 3 c 3 and 4.).....	50
Table 2-10 <i>P</i> value of the mean difference between control plants and inoculated plants for wet shoot weight, dry shoot weight, wet root weight and dry root weight from different pathogenicity test experiments (May 2003 and August 2003) under different temperatures (15°C, 25°C and 35°C) for <i>Rhizoctonia</i> sp. isolate. Means for the control plants were greater than the means for the inoculated plants. (Appendix 3 c 5.)	51
Table 2-11 <i>P</i> value of the mean difference between control plants and inoculated plants for wet shoot weight, dry shoot weight, wet root weight and dry root weight from different pathogenicity test experiments (November 2003, January 2004 and March 2004) under different temperatures (15°C, 25°C and 35°C) for the combination of <i>Phytophthora</i> sp. and <i>Pythium</i> sp. isolates. Means for the control plants were greater than the means for the inoculated plants. <i>P</i> values indicating significant differences ($P \leq 0.05$) are shown in bold. (Appendix 3 c 7.).....	51
Table 3-1 The four hydroponic lettuce farms surveyed in this study, including location, seedling sources and water source for nutrient solution.	64
Table 3-2 <i>P</i> values ($P \leq 0.05$ in bold) of the mean differences in root disease assessment between survey times, for the lettuce cultivar Baby Cos at four farms. (Appendix 4 b 1.) Shaded areas represent comparisons between cool (May-Aug) and warm (Nov-Jan-Mar) times.....	75
Table 3-3 <i>P</i> values ($P \leq 0.05$ in bold) of the mean differences in leaf symptom assessment between survey times, for the lettuce cultivar Baby Cos at four farms. (Appendix 4 b 2.) Shaded areas represent comparisons between cool (May-Aug) and warm (Nov-Jan-Mar) times.....	76

Table 3-4 <i>P</i> values ($P \leq 0.05$ in bold) of the mean differences in root disease assessment between survey times, for the lettuce cultivar Red Oak at four farms. (Appendix 4 c 1.) Shaded areas represent comparisons between cool (May-Aug) and warm (Nov-Jan-Mar) times.....	78
Table 3-5 <i>P</i> values ($P \leq 0.05$ in bold) of the mean differences in leaf symptom assessment between survey times, for the lettuce cultivar Red Oak at four farms. (Appendix 4 c 2.) Shaded areas represent comparisons between cool (May-Aug) and warm (Nov-Jan-Mar) times.....	79
Table 3-6 <i>P</i> values ($P \leq 0.05$ in bold) of the mean differences in root disease assessment between survey times, for the lettuce cultivar Green Oak at four farms. (Appendix 4 d 1.) Shaded areas represent comparisons between cool (May-Aug) and warm (Nov-Jan-Mar) times.....	81
Table 3-7 <i>P</i> values ($P \leq 0.05$ in bold) of the mean differences in leaf symptom assessment between survey times, for the lettuce cultivar Green Oak at four farms. (Appendix 4 d 2.) Shaded areas represent comparisons between cool (May-Aug) and warm (Nov-Jan-Mar) times.....	82
Table 3-8 <i>P</i> values ($P \leq 0.05$ in bold) of the mean differences in root disease assessment between survey times, for the lettuce cultivar Brown Mignonette at four farms. (Appendix 4 e 1.) Shaded areas represent comparisons between cool (May-Aug) and warm (Nov-Jan-Mar) times....	84
Table 3-9 <i>P</i> values ($P \leq 0.05$ in bold) of the mean differences in leaf symptom assessment between survey times, for the lettuce cultivar Brown Mignonette at four farms. (Appendix 4 e 2.) Shaded areas represent comparisons between cool (May-Aug) and warm (Nov-Jan-Mar) times....	85
Table 3-10 Number of lettuce plants sampled yielding <i>Pythium</i> species (n=5) at five sampling times from four cultivars at four hydroponic farms (except for CC2 farm where three cultivars were sampled in November 2004, January 2005 and March 2005). Nutrient solution temperature was measured in hydroponic channels on the sampling day. Totals show number of plants yielding <i>Pythium</i> compared with total numbers of plants sampled.	87
Table 3-11 Number of lettuce plants sampled yielding <i>Phytophthora</i> species (n=5) at five sampling times from four cultivars at four hydroponic farms (except for CC2 farm where three cultivars were sampled in November 2004, January 2005 and March 2005). Nutrient solution temperature was measured in hydroponic channels on the sampling day. Totals show number of plants yielding <i>Phytophthora</i> compared with total numbers of plants sampled.	88
Table 3-12 <i>P</i> values ($P \leq 0.05$ in bold) of the mean differences in root disease assessment between five observation times (days after inoculation) for Brown Mignonette lettuce inoculated with <i>Pythium</i> sp. (See also Figure 3-16.) (Appendix 4 g.)	91
Table 3-13 <i>P</i> values ($P \leq 0.05$ in bold) of the mean differences in root disease assessment between five observation times (days after inoculation) for non-inoculated (control) Brown Mignonette lettuce. (See also Figure 3-16.) (Appendix 4 g.).....	91
Table 3-14 Mean wet and dry weights (g) of roots and shoots of lettuces inoculated with <i>Pythium</i> and non-inoculated (control) lettuces. Within a column, means followed by the same letter are not significantly different from each other at $P \leq 0.05$. (Appendix 4 g.)	91
Table 4-1 Pathogens used in the three treatments involving inoculation of Brown Mignonette lettuce in the experiments at four different nutrient solution temperatures (with three experiments per temperature regime).	100
Table 4-2 Mean wet and dry weights (g) of roots and shoots at nutrient solution temperatures of 24-27°C after 21 days with four treatments (three inoculated and one control). Within a column, means followed by the same letter are not significantly different from each other at $P \leq 0.05$. For each mean value, n = 30. (Appendix 5 a.).....	105
Table 4-3 Average disease index over 3 to 21 days for root assessment of Brown Mignonette lettuce cultivar at nutrient solution temperatures of 24-27°C with four treatments (three inoculated and one control). Within a column, means followed by the same letter are not significantly different from each other at $P \leq 0.05$. (Appendix 5 a.)	105
Table 4-4 Mean wet and dry weights (g) of roots and shoots at nutrient solution temperatures of 34°C (continuously heated) after 21 days with four treatments (three inoculated and one control). Within a column, means followed by the same letter are not significantly different from each other at $P \leq 0.05$. For each mean value, n = 30. (Appendix 5 b.).....	108
Table 4-5 Average disease index over 3 to 21 days for root assessment of Brown Mignonette lettuce cultivar at nutrient solution temperatures of 34°C with four treatments (three inoculated and one control). Within a column, means followed by the same letter are not significantly different from each other at $P \leq 0.05$. (Appendix 5 b.).....	108
Table 4-6 Mean wet and dry weights (g) of roots and shoots at nutrient solution temperatures of 16-17°C after 21 days with four treatments (three inoculated and one control). Within a column, means followed by the same letter are not significantly different from each other at $P \leq 0.05$. For each mean value, n = 30. (Appendix 5 c.).....	111

Table 4-7 Average disease index over 3 to 21 days for root assessment of Brown Mignonette lettuce cultivar at nutrient solution temperatures of 16-17°C with four treatments (three inoculated and one control). Within a column, means followed by the same letter are not significantly different from each other at $P \leq 0.05$. (Appendix 5 c.)	111
Table 4-8 Mean wet and dry weights (g) of roots and shoots at nutrient solution temperatures of 34°C for 10 h followed by 18-20°C for the remainder of the 21 days with four treatments (three inoculated and one control). Within a column, means followed by the same letter are not significantly different from each other at $P \leq 0.05$. For each mean value, $n = 30$. (Appendix 5 d.)	115
Table 4-9 Average disease index over 3 to 21 days for root assessment of Brown Mignonette lettuce cultivar at nutrient solution temperatures of 34°C for 10 h followed by 18-20°C for the remainder of the 21 days with four treatments (three inoculated and one control). Within a column, means followed by the same letter are not significantly different from each other at $P \leq 0.05$. (Appendix 5 d.)	115
Table 5-1 Sequences of primers used for PCR amplification of the ITS region of rDNA.	130
Table 5-2 The restriction enzymes used for digestion of the ITS region of rDNA of <i>Pythium</i> , together with their recognition sequences and the incubation temperature and time.	131
Table 5-3 Sequences of primers used for PCR amplification for the ISSR analysis of <i>Pythium</i> isolates.	133
Table 5-4 Details of isolates of <i>Pythium</i> obtained from roots of different hydroponic lettuce cultivars during two farm-based surveys carried out in and near Sydney involving one farm in the first survey (Leppington 1; see Chapter 2) and four farms in the second survey (Leppington 1 and 2, and Central Coast 1 and 2; see Chapter 3).	135
Table 5-5 Mean growth (mm) in 24 h of 15 isolates of <i>Pythium</i> incubated at nine different temperatures from 5°C to 45°C on PCA in the dark. Growth was measured between 24 and 48h. Within a column, means followed by the same letter are not significantly different from each other at $P \leq 0.01$. $n = 5$ for each isolate at each temperature. (Appendix 7 a.)	144
Table 5-6 Sequence length (bp) of ITS region of rDNA for 13 isolates of <i>Pythium</i> (Appendix 7 b) from hydroponic lettuce roots and comparison with sequences in GenBank.	147
Table 5-7 Groups of isolates based on analysis of polymorphic fragments generated by AC ISSR primer. Isolates in bold are shown in Figure 5-9.	149
Table 5-8 Groups of isolates based on analysis of polymorphic fragments generated by AG ISSR primer. Isolates in bold are shown in Figure 5-10.	151
Table 5-9 Groups of isolates based on analysis of polymorphic fragments generated by CGA ISSR. Isolates in bold are shown in Figure 5-11.	153
Table 5-10 Groups of isolates based on analysis of polymorphic fragments generated by GT ISSR primer. Isolates in bold are shown in Figure 5-12.	155
Table 6-1 The restriction enzymes used for digestion of the ITS region of rDNA of <i>Phytophthora</i> , together with their recognition sequences and the incubation temperatures and time.	170
Table 6-2 Sequences of primers used for PCR amplification for the ISSR analysis of <i>Phytophthora</i> isolates.	170
Table 6-3 Details of isolates of <i>Phytophthora</i> obtained from roots of different hydroponic lettuce cultivars during two farm-based surveys carried out in and near Sydney involving one farm in the first survey (Leppington 1; see Chapter 2) and four farms in the second survey (Leppington 1 and 2, and Central Coast 1 and 2; see Chapter 3).	174
Table 6-4 Sequence length (bp) of ITS region of rDNA for four isolates of <i>Phytophthora</i> (Appendix 8 a) from hydroponic lettuce roots and comparisons with sequences in GenBank.	179
Table 6-5 Groups of isolates based on analysis of polymorphic fragments generated by AC ISSR primer. Isolates in bold are shown in Figure 6-5.	180
Table 6-6 Groups of isolates based on analysis of polymorphic fragments generated by AG ISSR primer. Isolates in bold are shown in Figure 6-6.	182
Table 6-7 Groups of isolates based on analysis of polymorphic fragments generated by CGA ISSR primer. Isolates in bold are shown in Figure 6-7.	184
Table 6-8 Groups of isolates based on analysis of polymorphic fragments generated by GT ISSR primer. Isolates in bold are shown in Figure 6-8.	186
Table 6-9 Sporangial size (length, breadth and length : breadth ratio) in 15 isolates of <i>Phytophthora drechsleri</i> isolated from roots of hydroponic lettuce. Within a column, means followed by the same letter are not significantly different from each other at $P \leq 0.05$. $n = 5$ for each isolate at each temperature. (Appendix 8 b.)	192

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BGT	Botanic Gardens Trust
bp	base pair
BSA	Bovine serum albumin
CLA	Carnation Leaf Agar
cm	centimetre
CMA	Corn Meal Agar
dATP	deoxyadenosine triphosphate
dCTP	deoxycytosine triphosphate
DI	Disease Index
DNA	deoxyribonucleic acid
dTTP	deoxythymidine triphosphate
EDTA	ethylenediaminetetraacetic acid
g	gram
GLM	General Linear Model
h	hour
ha	hectare
ISSR	Inter Simple Sequence Repeats
ITS	Internal Transcribed Spacer
min	minute
mL	millilitre
mm	millimetre
NFT	Nutrient Film Technique
ng	nanogram
PCA	Potato Carrot Agar
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PPA	Peptone PCNB Agar
PSA	<i>Phytophthora</i> Selective Agar
PYSA	<i>Pythium</i> Selective Agar
rDNA	ribosomal DNA
RFLP	Restriction Fragment Length Polymorphisms
rpm	revolutions per minute
s	second
SNA	Spezieller Nährstoffarmer Agar
U	Unit
UTS	University of Technology Sydney
UV	Ultraviolet
WA	Water Agar
μL	microlitre
μm	micrometre